Habitat Effects on Population Density and Movement of Insect Vectors of *Xylellafastidiosa* in California, USA

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ABSTRACT

Xylella fastidiosa is a xylem-limited bacterium that causes disease in grapevines, almonds, citrus, pear, alfalfa, and many other economically important plants. In California, USA, the bacteria are transmitted by several species of leafhoppers including the cicadellids *Draeculacephala minerva* Ball and *Homalodisca vitripennis* (Germar), the glassy-winged sharpshooter (GWSS). The pathogen and vectors have a wide host range including natural vegetation, cultivated crops, and ornamental plants in urban areas. Management of the diseases caused by *X. fastidiosa* requires knowledge of all possible infection pathways and biotic and abiotic factors that affect primary and secondary spread of the pathogen into and within agricultural landscapes. Two field studies were conducted to (*i*) determine patterns of insect vector population dynamics and temporal distribution of *X. fastidiosa*-infected plants relative to host plant assemblages in natural and cultivated habitats, and (*ii*) quantify movement and net dispersal rates of insect vectors in a manipulated experimental area. The first study investigated the role of *D. minerva* on movement of *X. fastidiosa* from different habitats into commercial almond nurseries, whereas the second study investigated the effects of deficit irrigated citrus trees on the spatiotemporal distribution and net dispersal rates of GWSS within the orchard. Surveys near commercial nurseries revealed that only habitats with permanent grass cover sustained *D. minerva* populations throughout the

season. A total of 87 plant samples tested positive for *X. fastidiosa* (6.3%), with a higher number of *X. fastidiosa*-infected plants found in weedy alfalfa fields than in other habitat types. Among plant species infected by *X. fastidiosa*, 33% were winter annuals, 45% were biennials or perennials, and 22% were summer annuals. Collectively, these findings identified a potential pathway for *X. fastidiosa* infection of almonds in nursery situations. Sex-specific net dispersal rates showed that GWSS males and females moved consistently and contributed equally to the level of population change within the citrus orchard. Trees under severe water stress were the least preferred by GWSS and yet, ca. 80% of the population were inflow individuals. Movement towards less preferable plants indicates that in agricultural landscapes dominated by perennial monocultures, there is a random component to GWSS movement, which may result from the inability of GWSS to use plant visual and/or olfactory cues to make well-informed long-range decisions.

Keywords: *Homalodisca vitripennis*, *Draeculocephala minerva*, plant water stress

INTRODUCTION

The glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), is an invasive insect pest native to the southeastern United States and northeastern Mexico [53] that was first discovered in California in the late 1980's [51]. The establishment of GWSS in California represents a serious threat due to its ability to vector *Xylella fastiodosa* Wells et al., a xylem-limited bacterium that causes Pierce's Disease in grapes [8], almond leaf scorch disease (ALSD) [9, 37], and many other diseases in economically important woody crops.

Since its initial detection, GWSS has expanded its range in Southern California and can also be found in southern portions of the San Joaquin Valley [3] and Pacific islands such as French Polynesia, Hawai'i, and Easter Island [17]. Pierce's disease affects grapevine (*Vitis vinifera* L.) production in the western and southeastern USA, whereas ALSD is found throughout almond production areas of California. Strains of *X. fastidiosa* are transmitted by several other species of xylem sap-feeding insects [13, 22, 42, 46, 47], but *Draeculacephala minerva* Ball (Hemiptera: Cicadellidae) is perhaps the only species that plays a role in pathogen spread to almond in California, as the distribution of *D. minerva* overlaps with almond production regions. GWSS is a polyphagous leafhopper with over 100 known hosts [25, 54]. GWSS populations are strongly associated with citrus plantings in California. Infested citrus orchards can

act as a source of vectors to adjacent vineyards as a result of the movement of GWSS between these two crops [4], which affects Pierce's disease incidence [39].

Effective management of a disease requires knowledge of all infection pathways. Proximity of susceptible crops to insect vector habitats is known to affect incidence of Pierce's disease in vineyards [16]. In almond orchards, in contrast, the random distribution of symptomatic trees and the absence of distinct disease gradients associated with adjacent vector habitat [18, 42] demonstrate that the relationships among proximity to vector habitat, the distribution of vectors in the orchard, and disease incidence are not as clear. Nonetheless, it is known that *D. minerva* moves between almond orchards and adjacent pastures and alfalfa fields [32], and the pathogen is present in *D. minerva* and in vegetation both in and around almond orchards [9]. Clearly, this species is an important vector of *X. fastidiosa* strains causing ALSD, and it may be responsible for some level of primary pathogen spread into orchards. Alternatively, another route of primary pathogen spread could be from infected nursery stock at the time of orchard establishment. Infection may occur in nurseries, either by the use of infected bud wood or transmission of the pathogen by insect vectors from surrounding vegetation into the nursery. Work by Hutchins et al. [27], Mircetich et al. [37], and Boyhan et al. [5] showed that *X. fastidiosa* can be transmitted by grafting in peach, almond, and plum, respectively. To our knowledge, primary spread of *X. fastidiosa* through the planting of infected almond nursery stock has not been considered.

Irrigation is the most significant input in agrosystems in arid and semi-arid regions worldwide. In California, future climate projection models predict reduced water reservoir carryover storage, reduced water availability to farmland in the Western San Joaquin Valley, and increased groundwater pumping [10]. Consequently, studies have developed water-saving strategies such as regulated deficit irrigation to improve water-use efficiency and sustainability in numerous perennial crop systems such as almonds [14, 52], citrus [15], and grapevines [55]. Regulated deficit irrigation is a strategy to maximize water use efficiency by reducing irrigation during drought-tolerant growth stages of a plant.

A significant amount of research has been generated to characterize the impact of plant stress on insect outbreaks and regulation of insect population dynamics. In general, resulting responses often appear to be insect feeding-guild dependent [33].

In this manuscript, results from two field studies, conducted separately, are

presented to illustrate the effects of habitat characteristics on population density and movement of insect vectors of *X. fastidiosa* in California, USA. In the first study with *D. minerva*[30], the objective was to evaluate the risk of infection of almond nursery stock from outside sources by quantifying vector populations and pathogen infection in host plant assemblages in habitats surrounding commercial almond nursery growing grounds. The hypothesiswas that natural vegetation in and around nursery plots included hosts for both *X. fastidiosa* and insect vectors. In the second study with GWSS [29, 31], the objective was to investigate the effects of deficit irrigation regimes in citrus trees on the population dynamics of GWSS. Results from the latter study demonstrated a relationship between GWSS population density and host plant quality, as measured by degree of water stress. However, differences in insect density among irrigation treatments may be a result of several mechanisms that act independently or in concert including differences in insect performance (e.g., fecundity and longevity) among irrigation treatments and differences in rates of movement based on treatment. Therefore, another goal of this study was to assess the extent to which movement affected GWSS population density and structure among irrigation treatments. Quantification of movement of GWSS was achieved through the combination of a mark-capture technique using multiple immunomarkers[19] and manipulation of irrigation levels in the orchard thereby inducing movement of marked GWSS individuals within the spatially heterogeneous habitat.

MATERIALS AND METHODS

Draeculocephala minerva **in habitats surrounding almond nurseries.**

Monitoring of *D. minerva* population dynamics. The population dynamics of *D. minerva* in vegetation located in and around commercial almond nurseries was monitored for one year. During this period, sharpshooter activity was monitored using yellow sticky traps placed around the perimeter of five almond nursery blocks. Seven common vegetation types (habitats) located adjacent to nursery blocks (10 to 15 m) were selected for sampling: irrigated pasture, drainage ditch, alfalfa field, weedy alfalfa field, non-cultivated perimeter, orchard floor, and cover crop. Throughout the trapping period, insect population densities in surrounding vegetation were monitored by collecting sweep net samples every six weeks.

Incidence of *X. fastidiosa* and plant species composition in *D. minerva* habitats. At each site, the relative cover and abundance of plant species were measured in 10 transects in the habitats described above. Linear transects were placed paralleled to the nursery blocks at ~ 14 m from the edge of the crop. Plant species composition within linear transects (10 m long × 0.3 m wide) was measured every six weeks for one year by recording species richness and species diversity (Simpson's Index of Diversity) [(49)]. At each sampling date, leaf samples were collected from plant species present within each transect and tested for presence of *X. fastidiosa* using an ELISA kit.

GWSS population density and dispersal in a water-stressed citrus orchard block

Experimental site and irrigation treatments. A two year study was conducted on the campus of the University of California, Riverside, in 5.4 ha of a citrus orchard [*Citrus sinensis* cv. 'Valencia'] maintained under micro-sprinkler irrigation. The experiment was designed as a 3 x 3 Latin square with three irrigation treatments: 1) trees irrigated at 100% of the crop evapotranspiration rate (ET_c), 2) a continuous deficit-irrigated treatment maintained at 80% ET_c, and 3) a continuous deficit-irrigated treatment maintained at 60% of ET_c throughout the two years of the experiment. Each of the nine plots consisted of 120 trees (23.6 m^2 canopy cover).

Plant conditions. The severity of water stress was characterized weekly by measurements of stem water potential using a pressure chamber. To monitor the water potential, the fourth leaf from the tip of two mature branches per tree was covered with a bag made of foil-laminate material for 30 min before being excised from the branch. Leaves were excised and immediately processed.

Fruit quality and yield. All oranges were harvested and taken to a local commercial packing house where oranges were mechanically counted, sized, and color graded. Fresh market oranges were categorized as "first" (higher quality) or "second" (lower quality) grade.

GWSS populations. Populations of GWSS within experimental plots were sampled weekly for two years. A 3-min visual inspection of leaves and branches around sample trees was conducted to monitor for GWSS egg masses, nymphs, and adults. The same trees were sampled for GWSS adults and nymphs by collecting a beat net sample from each tree. Yellow sticky traps were used to monitor insect

activity. Six traps were placed on the south side of three rows per plot (two traps per row placed five trees apart). Traps were replaced weekly and placed into a freezer until inspection.

Mark and capture of GWSS. Three unique proteins were used in the study including cow's milk (casein), chicken egg white (egg albumin), and soy milk (soy trypsin) to mark GWSS in the 60, 80, and 100% ET_c treatments, respectively. Homogenized whole milk, chicken egg white, and soy milk were purchased from local wholesale distributors and stored at 4°C until use. On the application date, each of the marking materials were diluted in water to a 5% solution and applied to trees in the respective treatment plots at a rate of 1870.6 l / ha using a tractor PTO-driven, airblast sprayer. Applications were repeated on three different dates in 30-day intervals starting in late-June and ending late-August in each year of the study. Yellow sticky traps deployed as described above were used tomonitor insect activity. GWSS adults were removed from the traps and placed into individual 1.5-mlvials for ELISA analysis.

ELISA for marker detection. A bovine casein, egg albumin, and soy trypsin indirect ELISA was performed on field-captured GWSS as described in detail by Jones et al. [(28)] to determine the captured individual area of origin.

Net dispersal rate of GWSS. A weekly, sex-specific net dispersal rate (NDR) of GWSS for each irrigation treatment was calculated as the ratio of the difference between the number of inflow and outflow individuals to the number of residents, as follows:

$$NDR = (i - o)/r \quad [1]$$

where the number of residents (r) was the number of insects caught in the reference irrigation treatment that were ELISA-positive only for the protein marker applied to the reference irrigation treatment. The number of inflow individuals (i) was the number of insects caught in the reference irrigation treatment that were ELISA-positive for one or both of the protein markers applied to the other irrigation treatments. Finally, the number of outflow individuals (o) was the number of insects caught outside the reference irrigation treatment that were ELISA-positive for the protein marker applied to the reference irrigation treatment. Positive values for NDR indicate that more GWSS entered an irrigation treatment than left, whereas negative values for NDR indicate that more GWSS left an irrigation treatment than entered. The impact of such movement on population composition was measured relative to the size of the resident population. Individuals that were ELISA-positive for two or three markers were not included in the number of outflow individuals because their origin was unknown.

RESULTS

Draeculocephala minerva in habitats surrounding almond nurseries.

Monitoring of insect vector population dynamics. A total of 22 species of Cicadomorphs were collected in sweep net samples. Of these, *D. minerva* was the only known vector of *X. fastidiosa* captured. The numbers of *D. minerva* adults captured were highest on the edges of irrigated pastures, followed by drainage ditches and edges of weedy alfalfa fields (Fig. 1A). Insect population densities in weedy alfalfa fields were about three-fold higher than in weed-free alfalfa fields.

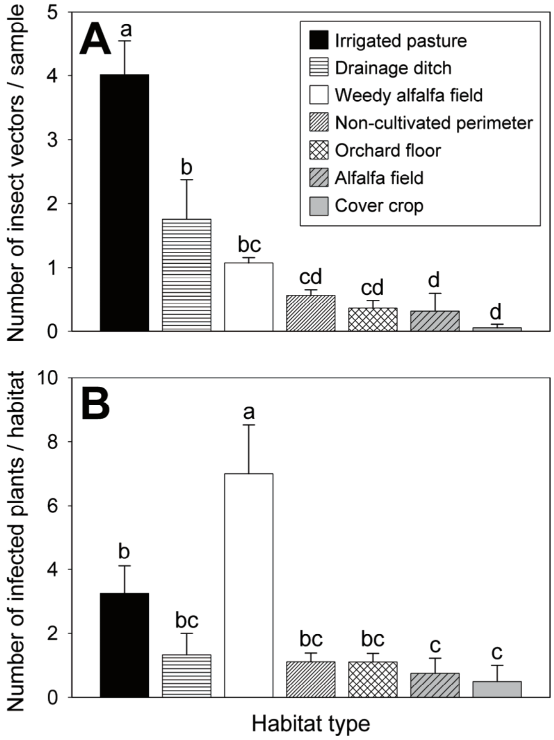

Fig. 1. Mean (± SEM) numbers of *Draeculacephala minerva* adults in sweep net samples collected from vegetation in habitats surrounding almond nursery grounds, **A**, and mean number of *Xylella fastidiosa*-infected plants per habitat, **B**. Bars representative of habitat type having the same letter above them do not differ significantly ($P< 0.05$) according to a Tukey's HSD test.

There was a curvilinear relationship between the numbers of *D. minerva* and the percentage of cover by grass species in sampled habitats (Fig. 2), such that the numbers of

insects caught in the samples increased with increasing grass cover. Although some habitats referred to here as non-cultivated perimeter, orchard floor, and cover crop had a high percentage of grass cover during winter and spring months, only habitats with permanent grass cover (i.e., irrigated pastures and drainage ditches) were shown to sustain robust *D. minerva* populations throughout the season.

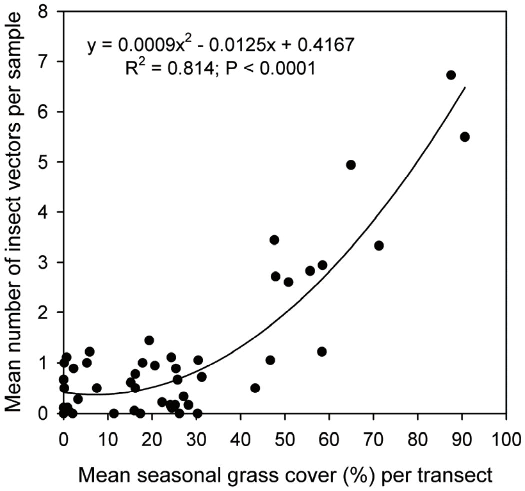

Fig. 2. Relationship between the mean (± SEM) numbers of *Draeculacephala minerva* adults captured in sweep net sampling and the mean seasonal grass cover on habitats surrounding almond nursery grounds.

Insect catch data from yellow sticky traps, when pooled across all habitats, showed five peaks of *D. minerva* adult activity throughout the sampling period (Fig. 3). Traps located on the edge of surrounding habitats consistently captured more *D. minerva* adults than traps located on the edge of nursery stock growing grounds. Despite the reduced insect activity from mid- March to early May, trap catches within nursery stock grounds indicated that *D. minerva* adults were actively moving between the surrounding vegetation and the nursery crop.

Incidence of *X. fastidiosa* and plant species composition in vector habitats. A total of 102 plant species were identified and 1387 samples were collected. A total of 87 samples tested positive for *X. fastidiosa* (6.3%) with a higher number of infected plants found inweedy alfalfa fields than in the other habitat types (Fig. 1B).

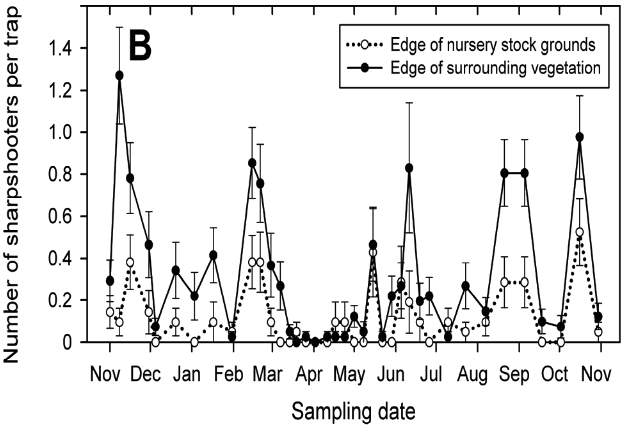

Fig. 3. Mean (± SEM) numbers of *Draeculacephala minerva* adults captured in yellow sticky traps placed between nursery growing grounds and surrounding vegetation. Bars representative of sampling dates having the same letter above them do not differ significantly (*P*< 0.05) according to a Tukey's HSD test.

Measurements of plant species richness and species diversity showed that alfalfa fields and drainage ditches were the least and the most rich and diverse habitats, respectively. The mean (± SEM) plant species richness in alfalfa fields and drainage ditches was 1.133 ± 0.133 and 5.778 ± 1.806 species per transect per sampling period, respectively. Species diversity (Simpson's Index of Diversity) in alfalfa fields and drainage ditches was 0.004 ± 0.004 and 0.569 ± 0.161 per transect per sampling period, respectively. Values of species richness and diversity for irrigated pastures and weedy alfalfa fields were intermediate among the habitats. On average (± SEM), plant species richness in irrigated pastures and weedy alfalfa fields was 3.62 ± 0.73 and 4.22 ± 1.13 species per linear transect per sampling period, respectively. Species diversity values in irrigated pastures and weedy alfalfa fields were 0.225 ± 0.081 and 0.287 ± 0.082 per linear transect per sampling period, respectively.

Although measurements of plant species richness and diversity within habitats did not markedly vary throughout the sampling period, plant species composition in habitats changed according to plant species' life cycle (e.g., annual vs. perennial) and seasonality (e.g., winter vs. summer). Among the 40 plant species that tested positive for *X. fastidiosa*, about one third were winter annuals, one third were biennials or perennials, and one third were summer annuals that accounted for about 33.3, 44.8, and 21.8% of all *X. fastidiosa*-positive plants, respectively (Table 1). Although the majority of the *X. fastidiosa*-positive plant species reported here had been reported as

hosts in previous surveys, a total of 19 new plant species are reported here as potential hosts of *X. fastidiosa*. Among the sampling dates, *X. fastidiosa* detection was highest during the month of February, followed by July. There were no significant differences in proportion of infected plants among the other sampling dates.

GWSS population density and dispersal in a water-stressed citrus orchard

Plant conditions. Stemwater potential was consistently lower in the 60% ET_c treatment than in the 80 or 100% ET_c treatments. There were no differences in stem water potential between the 80 and 100% ET_c treatments.

Fruit quality and yield. In 2006, there were no differences in total numbers of harvested fruit or in the number of fruit per grade category among irrigation treatments. However, the percentage of first grade fruit was higher in the 80% ET_c treatment. Moreover, the percentage of first grade fruit was significantly lower in the 60% than in the 100% ET_c treatment. There were no differences in the percentages of low quality, non-juice (second grade) fruit among treatments. In 2007, the total number of harvested fruit in the 60% ET_c treatment was significantly lower than in the 80 and 100% ET_c treatments. The numbers of fruit across all fruit grade categories were lower in the 60% ET_c treatment than in the 80 and 100% ET_c irrigation treatments. There were no differences in total number of fruit and number of fruit per grade category between the 80 and 100% ET_c irrigation treatments.

GWSS populations. Visual counts in 2005 revealed an increase in adult GWSS levels from late June to a peak in mid July. During this period, about 50% fewer adults were counted on trees irrigated with 60% of the ET_c than with 80 and 100% ET_c. There was no difference in the number of GWSS adults observed per tree between the 80 and 100% ET_c treatments. In 2006, there was an increase in the overall number of adult GWSS observed per tree in early July to the population peak in late July. Up to the peak of GWSS numbers in late July, fewer adults were found on trees irrigated at 60% ET_c than at 80 and 100% ET_c. There was no difference in the number of GWSS adults observed per tree between 80 and 100% ET_c treatments. Averaging over the early July to early October interval, fewer adult GWSS were found in trees irrigated at 60% ET_c than at 80% ET_c. The number of adult GWSS counted in the 100% ET_c treatment was not different from those observed in the 60% or 80% ET_c irrigation treatments.

During the 2005 sampling period, there were two peaks of GWSS oviposition (mid-May and mid-July). However, there were no differences in the mean number of GWSS egg masses observed among the irrigation treatments throughout either the

mid-April to mid-May interval or during the second, and highest, egg mass peak lasting from mid-June to early August. In 2006, there appeared to be four discrete periods of GWSS oviposition. The first period, resulting from oviposition by overwintering adults, occurred from late February to early March. A second peak occurred from late April to early June, and the third and largest peak occurred from early July to early September. The fourth discrete oviposition period occurred from late September to late October. In only one of the four periods were any differences in GWSS egg masses observed as a result of deficit irrigation treatment. Specifically, fewer egg masses were found in the 60% than in the 80 and 100% ET_c treatments during the second peak ovipositional period of 2006.

Sex-specific net dispersal rate of GWSS. Male and female GWSS NDRs were similar and followed the same trend during the 2005 and 2006 seasons (Fig. 4). Weekly NDRs calculated for each irrigation treatment showed that inflow movement (i.e., individuals moving into a block) was consistently higher than outflow movement (i.e., individuals moving out of a block) in the 60% ET_c (2005 and 2006) and 100% ET_c treatments (2005 only). NDRs were generally neutral in the 80% ET_c treatment in both years (Figs. 4C and 4D) and neutral in the100% ET_c treatments in 2006 only (Fig. 4F). In the 100% ET_c treatment, inflow and outflow movements were the same except for the period of 24 July to 7 August 2006 when the number of inflow individuals exceeded the number of outflow individuals (Fig. 4F).

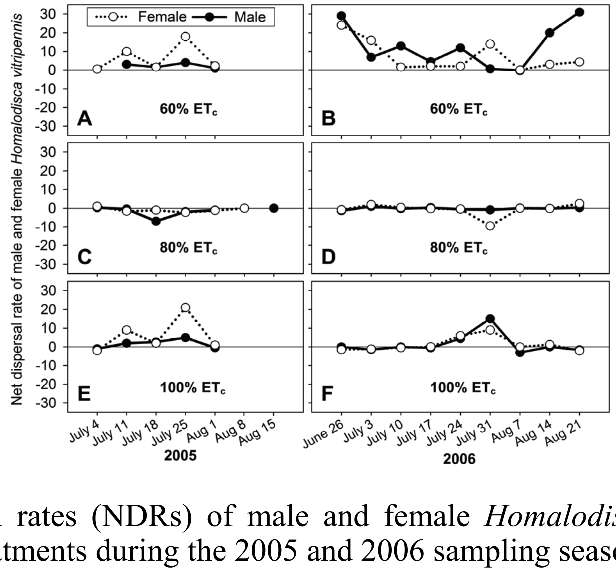

Fig. 4. Net dispersal rates (NDRs) of male and female *Homalodisca vitripennis* in irrigation treatments during the 2005 and 2006 sampling seasons obtained from data on number of dispersing individuals captured on traps. NDRs were calculated using equation 1. Positive and negative NDRs in the 60% (Fig. A and B) and 80% ET_c irrigation treatments (Fig. C and D) show higher inflow and outflow movement, respectively.

Ignoring gender, more individuals moved into the 60% ET_c treatment than moved out of the 60% ET_c treatment in 2006. Conversely, more individuals moved out of the 80% ET_c and 100% ET_c treatment areas than moved into the 80% ET_c and 100% ET_c treatments. Resident populations peaked on 24 July in the 80% ET_c treatment and were overall higher than resident populations in the other irrigation treatments.

Composition of GWSS populations. The composition of GWSS populations within irrigation treatments was similar during the 2005 and 2006 seasons. In 2005, inflow individuals that originated from the 80% ET_c irrigation treatment were more abundant in the 60% ET_c (~51% of ELISA-positive insects) and 100% ET_c treatments (~65% of ELISA-positive insects) than residents and other inflow individuals (Table 2). In 2006, individuals that originated from the 80% ET_c treatment were also more abundant than the resident populations in the 60% ET_c (~27 vs. 12% of ELISA-positive insects) and 100% ET_c treatments (~55 vs. 37% of ELISA-positive insects, respectively) (Table 2). Resident populations in the 80% ET_c treatment were higher than the inflow populations (Table 2). Resident populations in the 60 and 100% ET_c treatments were in the minority (<50%) in both 2005 and 2006.

DISCUSSION

One of the goals of the study on *D. minerva* in almond nurseries was to investigate the potential role of infected nursery stock in contributing to ALSD occurrence in commercial almond orchards. Surveys conducted in vegetation found near commercial nursery growing grounds revealed that vector population densities and incidence of *X. fastidiosa* are highly dependent on vegetation type. As both vector and pathogen were found in close proximity to almond nurseries, spread of *X. fastidiosa* into nurseries is considered plausible.

In the past 60+ years, numerous studies have demonstrated the importance of non-crop plants species as potential sources of *X. fastidiosa*[2, 6, 12, 20, 21, 23, 24, 26, 35, 36, 43, 44, 45, 48, 50, 56]. Although most *X. fastidiosa* host plant species documented here have been reported as hosts in other surveys, 19 new plant hosts were identified.

Draeculacephala minerva is well known to be abundant in irrigated pastures, stream banks, and weedy alfalfa fields with perennial grass cover [7, 41, 50]; proximity of such habitats near almond orchards with high incidence of ALSD has been documented [40]. Results from surveys reported here, such as high vector abundance and presence of *X. fastidiosa* in host plants located adjacent to the crop, are in agreement with

findings from previous investigations. However, this study is the first to establish presence of vectors and *X. fastidiosa* specifically with almond nurseries.

Proximity of *X. fastidiosa* and insect vectors to commercial almond nurseries in California was demonstrated, providing evidence for *X. fastidiosa* infection of nursery stock. However, as ALSD incidence in California is typically low in almond orchards, primary spread via infected nursery stock also must be low under the current conditions. Nursery plants may not show symptoms of ALSD while in the nursery, which makes it impossible to use symptom expression for roguing prior to commercialization. Moreover, screening the large numbers of plants cultivated by commercial nurseries (12,000 plants/ ha) for presence of *X. fastidiosa* using assays such as culturing, PCR, or ELISA is impractical, laborious, and could result in the addition of unnecessary production costs. Therefore, removal and replacement of diseased plants soon after orchard establishment may be the most cost effective practice for both almond growers and almond nursery stock producers.

The study on the effects of citrus deficit irrigation on GWSS showed that the two irrigation deficit regimes, 60 and 80% ET_c, differentially affected the population dynamics of GWSS in the experimental citrus plots. GWSS populations were negatively affected by severe host plant water stress, but GWSS population density was not linearly correlated with decreasing water availability in plants. Trees irrigated at 60% ET_c were host to fewer GWSS eggs, nymphs, and adults than trees irrigated at 80% ET_c. Interestingly, the 100% ET_c treatment hosted similar numbers of GWSS eggs, nymphs, and adults as the 60% ET_c treatment in some periods of the study and lower numbers of GWSS nymphs than the 80% ET_c. Moderate water stress in trees (e.g., 80% ET_c) may increase solute concentrations used for osmotic adjustment (i.e., carbohydrates, amino acids, and organic acids) that may also serve as feeding stimulants and nutritional substrates [(34)]. However, reduced water potential beyond a certain threshold in more severely water-stress irrigation treatments (60% ET_c) might impede GWSS feeding because more energy would be needed to extract xylem fluid [(1)]. Conversely, well-watered plants (100% ET_c) with higher mean water potentials may facilitate extraction of xylem fluid. However, as the energy required for extracting xylem fluid was reduced in well-watered trees, more fluid would have to be ingested and filtered to compensate for a more dilute xylem food source. Thus, citrus trees irrigated at 80% ET_c may combine two important plant characteristics for GWSS: 1) a nutrient-concentrated food source and 2) a water potential above

acceptable thresholds for GWSS xylem fluid extraction.

Our data demonstrated that a water saving of 20% (i.e., irrigation at 80% ET_c) over two years did not induce significant reduction in yield and fruit quality compared to full irrigation (100% ET_c). The 20% water saving practice improved water use efficiency (yield per unit water) and thus, seems a viable option for commercial practice to maintain productivity and reduce irrigation costs in areas with scarce water resources. However, long term effects of this deficit irrigation regime on plant vegetative growth needs further investigation.

Findings from this study have generated significant new information regarding the host selection behavior of GWSS in California. Trees under severe water stress had lower water potential and consequently hosted fewer GWSS than trees maintained under moderate water stress. Although the adult GWSS population was reduced, on average, by 50 to 65% in citrus plots maintained under continuous severe water stress, the negative economic impacts to citrus growers reflected by lower yield and fruit quality (50% overall reduction), especially after two consecutives years of severe water stress, impedes the adoption of this management strategy to reduce GWSS populations in Valencia orchards in southern California. Nevertheless, regulated deficit irrigation (RDI) regimes are widely practiced over millions of hectares worldwide to reduce usage of irrigation water and increase farmer's profits [(11)]. A more complete understanding of the effect of RDI applied during less vulnerable phenological stages of citrus fruit development and the operative host-plant cues that influence GWSS host selection behavior may result in the deployment of strategies to improve control efforts.

Irrigation levels established in the experiment induced movement revealing the mobility of both male and female GWSS. The mark-capture method used in the current study aided our ability to document and track insect movement in the experimental area demonstrating the effects of movement on GWSS population density and structure among irrigation treatments. Sex-specific net dispersal rates showed that males and females moved consistently within the habitat and contributed equally to the overall level of population change within and among irrigation treatments. Resident GWSS represented the minority in the 60 and 100% ET_c irrigation treatments during the 2005 and 2006 seasons, whereas resident GWSS were in the majority in the 80% ET_c irrigation treatment in both seasons. Insects originating from the 80% ET_c treatment plots were also in the majority in the other irrigation treatments; except in 2006 when there was no difference in the percentage of inflow individuals in the 60%

ET_c treatment. A total of 5,970 and 10,478 individuals in 2005 and 2006, respectively, were tested for the presence of each protein mark. Of these, about 10 and 27% tested positive for at least one marker in 2005 and 2006, respectively. Despite the fact that the percentages of double- (or triple-) marked individuals were generally low compared to single-marked individuals, the presence of multi-marked individuals showed that movement of GWSS within the discrete habitat was not unidirectional. That is, between 0.4 and 1% of all ELISA-positive individuals had visited the three different marked areas before being captured.

Differential inflow and outflow movement within and among experimental treatments were the major factors contributing to the observed unbalanced distribution and composition of the population within the experimental area. Among all protein-marked individuals trapped in the 60% ET_c irrigation treatment, about 75 and 88% of them in 2005 and 2006, respectively, were inflow individuals. Movement towards less preferable host plants indicates that GWSS were unable to make well-informed decisions based on visual or olfactory cues in selecting suitable host plants and habitats during movement. These findings are in agreement with those of Northfield et al. [38] who reported movement of GWSS individuals from a high to low quality patch. Data also support their hypotheses that: 1) GWSS move into low quality patches from a distance, but leave the patch after assessing host plant quality such as xylem fluid quality, and 2) GWSS host selection behavior occurs on the plant rather than from plant visual or physical cues available prior to landing. Collectively, these studies demonstrate that there is a random component to GWSS movement and dispersal in agricultural landscapes dominated by perennial monocultures.

The overall goal of this study was to improve our understanding of the factors that: 1) influence GWSS movement in a managed ecosystem, 2) control fluctuating population densities within a manipulated habitat, and 3) reduce GWSS impact on affected crops. In addition to quantifying net dispersal rates and describing the composition of GWSS populations, this study is an important step towards predicting seasonal movement of GWSS according to habitat conditions.

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Table 1. Total number of infected plants over the number of sampled individuals on each collection date and their temporal distribution in habitats surrounding five nursery stock blocks

Plant species	Plant family	Sampling date					
		2/6/08	3/21/08	5/23/08	7/24/08	9/11/08	10/23/08
Bromus diandrus Roth[a]	Poaceae	1/1	d	d	d	d	d
Avena fatua L.	Poaceae	4/4	d	d	d	d	d
Hordeum murinum L. ssp. *murinum*	Poaceae	2/5	0/3	d	d	d	d
Erodium botrys (Cav.) Bertol.[a]	Geraniaceae	1/4	0/4	d	d	d	d
Lolium perenne L.[a]	Poaceae	5/5	c	d	d	d	d
Capsella bursa-pastoris (L.) Medik.[b]	Brassicaceae	1/4	0/3	0/2	d	d	d
Poa annua L.	Poaceae	3/6	c	c	d	d	d
Stellaria media (L.) Vill.	Caryophyllaceae	1/10	0/2	c	d	d	d
Senecio vulgaris L.	Asteraceae	2/5	1/8	0/1	d	d	d
Ranunculus repens L.[b]	Ranunculaceae	1/2	0/2	c	c	d	d
Cyperus eragrostis Lam.	Cyperaceae	1/1	0/1	c	c	d	d
Geranium dissectum L.[b]	Geraniaceae	0/3	0/3	c	1/1	d	d
Medicago polymorpha L.	Fabaceae	2/5	0/2	c	c	c	d
Lactuca serriola L.	Asteraceae	c	c	0/6	2/6	0/3	d
Verbena litoralis Kunth[b]	Verbenaceae	0/3	1/2	0/3	c	0/3	d
Silybum marianum (L.) Gaertn.[b]	Asteraceae	1/6	0/3	0/1	0/1	c	0/1
Erodium moschatum (L.)	Geraniaceae	1/22	0/13	0/1	c	0/1	c
Ludwigia grandiflora (Michx.)[b]	Onagraceae	0/3	1/12	c	c	0/6	c
Marrubium vulgare L.[b]	Lamiaceae	3/6	0/4	0/1	0/2	0/1	0/2
Medicago sativa L.	Fabaceae	7/26	0/23	1/30	0/26	0/31	0/32
Cynodon dactylon (L.) Pers.	Poaceae	c	c	2/9	1/2	1/7	0/4
Sonchus oleraceus L.	Asteraceae	1/2	0/12	0/8	1/5	0/1	0/2
Malva parviflora L.	Malvanaceae	7/29	0/17	0/10	2/6	0/11	0/16
Conyza canadensis (L.) Cronquist	Asteraceae	c	c	0/10	1/9	0/8	0/6
Rumex crispus L.	Polygonaceae	3/14	0/7	0/9	c	0/1	3/5
Coronopus didymus (L.) Smith[b]	Brassicaceae	1/10	c	0/1	0/2	c	1/1

Plantago lanceolata L.[b]	Plantaginaceae	0/2	0/1	0/2	0/3	0/3	1/3
Datura wrightii Regel	Solanaceae	d	0/8	0/25	4/18	0/24	0/34
Prunus dulcis (Mill.) D. A. Webb	Rosaceae	d	0/12	0/14	2/20	0/33	0/35
Vitis spp.	Vitaceae	d	0/2	0/2	1/2	0/2	0/2
Convolvulus arvensis L.	Convolvulaceae	d	d	0/6	2/10	0/3	0/2
Salsola tragus L.[b]	Chenopodiaceae	d	d	0/2	1/2	0/1	0/2
Eriochloa contracta Hitchc.[a]	Poaceae	d	d	d	1/1	c	d
Echinochloa crus-galli (L.) P. Beauv.	Poaceae	d	d	d	1/4	0/2	0/2
Polygonum arenastrum Jord.[a]	Polygonaceae	d	d	d	1/5	0/1	0/3
Polygonum lapathifolium L.[a]	Polygonaceae	d	d	d	1/5	c	0/3
Agrostis gigantea Roth[b]	Poaceae	d	d	d	0/1	c	1/5
Carex L.[b]	Cyperaceae	d	d	d	c	0/1	1/1
Xanthium spinosum L.[a]	Asteraceae	d	d	d	0/1	0/13	1/5
Portulaca oleracea L.	Portulaceae	d	d	d	2/8	d	d

[a] Congener species previously reported as a host.

[b] First report of *Xylella fastidiosa* detection in plant genus and species.

c No plant samples collected.

d Plant species not present in or near line transects.

Table 2. Accumulated percentage distribution of protein-marked GWSS in irrigation treatments during the 2005 and 2006 summer seasons

Irrigation treatment	Sex	Insects tested	Insects marked	Mean (± SE) percentage of protein-marked individuals					
				Casein[a] (60% ET_c)	Egg albumin (80% ET_c)	Soy trypsin (100% ET_c)	Casein + egg albumin[b]	Casein + soy trypsin	Egg albumin + soy trypsin
2005									
60% ET_c	♂	406	28	30.90 ± 9.25	52.12 ± 11.91	12.12 ± 9.29	-	-	4.84 ± 3.37
	♀	510	72	21.00 ± 7.66	49.17 ± 8.84	14.34 ± 4.64	-	-	15.47 ± 5.60
	Total	**916**	**100**	**25.19 ± 5.87 Ab**	**50.42 ± 7.02 Aa**	**13.4 ± 4.65 Ab**	**-**	**-**	**10.97 ± 3.63 Ab**
80% ET_c	♂	472	36	7.69 ± 7.69	58.49 ± 12.33	33.81 ± 11.68	-	0	-
	♀	595	60	8.29 ± 4.48	71.40 ± 7.62	20.30 ± 6.19	-	0	-
	Total	**1067**	**96**	**8.03 ± 4.11 Ac**	**65.81 ± 6.84 Aa**	**26.15 ± 6.16 Ab**	**-**	**0**	**-**
100% ET_c	♂	479	32	20.45 ± 12.06	63.63 ± 13.63	11.36 ± 6.18	4.54 ± 4.54	-	-
	♀	523	66	19.04 ± 9.86	65.67 ± 10.53	12.20 ± 7.34	3.07 ± 2.09	-	-
	Total	**1002**	**98**	**19.66 ± 7.49 Ab**	**64.77 ± 8.23 Aa**	**11.83 ± 4.83 Ab**	**3.72 ± 0.97 Ab**	**-**	-
				$F_{2,71} = 2.426$	$F_{2,71} = 0.981$	$F_{2,71} = 2.311$		$F_{1,43} = 2.933$	
				$P = 0.096$	$P = 0.380$	$P = 0.107$		$P = 0.094$	
2006									
60% ET_c	♂	1011	205	10.20 ± 3.31	26.42 ± 3.43	38.58 ± 4.38	-	-	24.79 ± 4.43
	♀	654	124	13.21 ± 2.81	27.07 ± 3.87	25.23 ± 4.68	-	-	34.47 ± 3.46
	Total	**1665**	**329**	**11.70 ± 2.15 Ab**	**26.75 ± 2.55 Ba**	**31.91 ± 3.35 Aa**	**-**	**-**	**29.63 ± 2.89 Aa**
80% ET_c	♂	1208	422	1.69 ± 0.75	62.11 ± 2.76	35.35 ± 2.85	-	0.83 ± 0.48	-
	♀	722	268	5.91 ± 1.51	52.12 ± 2.22	41.09 ± 2.92	-	0.85 ± 0.50	-
	Total	**1930**	**690**	**3.80 ± 0.91 Bc**	**57.12 ± 1.94 Aa**	**38.22 ± 2.07 Ab**	**-**	**0.84 ± 0.34 Bc**	**-**
100% ET_c	♂	1023	235	5.58 ± 1.51	52.45 ± 4.28	39.73 ± 4.24	2.22 ± 1.86	-	-
	♀	621	176	6.76 ± 2.57	57.64 ± 5.64	34.79 ± 5.25	0.80 ± 0.61	-	-
	Total	**1644**	**411**	**6.17 ± 1.47 Abc**	**55.05 ± 3.52 Aa**	**37.26 ± 3.35 Ab**	**1.51 ± 0.97 Bd**	**-**	**-**
				$F_{2,98} = 4.575$	$F_{2,98} = 39.359$	$F_{2,98} = 2.623$		$F_{2,98} = 126.998$	
				$P = 0.013$	$P < 0.001$	$P = 0.078$		$P < 0.001$	

[a] Casein, egg albumin, and soy trypsin, which were applied to irrigation treatments 60, 80, and 100% ET_c, respectively.
[b]GWSS ELISA-positive for two protein markers.
The same upper case letters in columns and lower case letters in rows indicate irrigation treatments and protein markers, respectively, did not significantly affect the number of captured and marked individuals ($\alpha = 0.05$).